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"SILVER-LEAF" DISEASE.

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William G. Smith.

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"SILVER-LEAF" DISEASE.

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Silver-leaf disease attacks several different kinds of fruit trees and various other plants. All available evidence tends to shew that this disease is becoming more and more serious each year. As the name implies, the foliage of affected plants presents a silvery appearance in contrast to the dark green colour of healthy leaves. At the commencement of attack a single branch alone usually shews signs of silvering, but in the course of a year or two the affection usually spreads throughout the tree; subsequently branches of the tree begin to die and finally the whole tree succumbs.

Percival (3) was the first to call serious attention to this disease. In 1902 he shewed that the silvery appearance of the leaves was due, not to any change in the constitution of the chlorophyll, but to the formation of air cavities in certain walls of the epidermal cells. Percival found that portions of roots of affected trees gave rise to the sporophores of *Stereum purpureum* when kept in a moist atmosphere. Upon inoculation of branches of healthy trees with portions of these sporophores, silvering of the foliage ensued. He concluded therefore that *Stereum purpureum* was the cause of the disease.

In 1906 Spencer Pickering (4) performed a number of inoculation experiments with the sporophores of this fungus, and in the large majority of cases silvering of the leaves resulted. More recently this investigator (5) has published further observations on the same disease in which he emphasises the active *rôle* played by *Stereum purpureum* in this disease. In the same publication Spencer Pickering shews that it is possible to cause silvering of Apple trees by inoculation with *Stereum purpureum* derived from Plum trees and silvering of Plum trees by inoculation with *Stereum* from a Laburnum, so it does not appear that this fungus is "specialised" to particular hosts. He mentions also the interesting fact that Plum trees occasionally recover from silvering and bear normal foliage again.

During the last three years I have been making observations and experiments in connection with this disease in Cambridgeshire, and though the work is not yet complete it has been thought desirable to publish some of the results already obtained. A preliminary account (1) of some of these observations was read at the meeting of the British Association in Sheffield in 1910. The work has been much facilitated recently by a grant which has been made to me by the Development Commissioners in order to provide fruit trees for experimental purposes; I should like to offer thanks for this assistance. I am also indebted to the Cambridge University School of Agriculture for the allocation of a plot of land at their Experimental Farm for the fruit trees provided by the grant already mentioned.

In Cambridgeshire as in other parts of the country, Plum trees are much more liable to Silver-leaf than any other kind of fruit tree. The varieties known as "Victoria" and "Czar," especially the former, are more susceptible to the disease than are other varieties. Plum trees are usually several years old before they are attacked, and I have only seen a single two-year-old tree that has become silvered without being artificially inoculated. A feature of the distribution of Silver-leaf on Plum trees is the great prevalence of the disease in small private gardens where only one or two Plum trees are present. Several plantations of Plum trees in Cambridgeshire contain more than 10 per cent. of silvered trees and in one commercial garden which has been neglected more than half the trees (Victorias) are attacked. By the kindness of Mr A. W. Hill and Mr G. Massee of the Royal Gardens, Kew, I recently had the opportunity of seeing in that district a plantation of Plum trees badly affected with Silver-leaf. In this case also more than half the Victorias were diseased and were dying rapidly.

Massee (2) notes the fact that the wild Blackthorn or Sloe sometimes becomes silvered in this country, and other species of the genus Prunus, including the Portugal Laurel, are also liable to Silver-leaf disease[1].

Nor is it only species of this genus which are liable to the malady. Apple trees are not uncommonly affected and in this district the scions of regrafted trees are often found to be silvered. Spencer Pickering (5) mentions a single Pear tree which became silvered after inoculation with *Stereum*, but I have not yet heard of Pear trees becoming attacked without such inoculation. During the discussion on Silver-leaf at the meeting of the British Association at Sheffield last year, Lieut.-Col.

[1] I recently saw a silvered Cherry tree at Odda, Hardanger Fiord, Norway.

Prain mentioned the silvering of a Spiraea in the Royal Gardens, Kew. I have seen a few Gooseberry and Red Currant bushes affected with Silver-leaf and records have previously been published of the occurrence of the disease on Laburnum. Spencer Pickering (5) refers to a correspondent in New Zealand who makes mention of a silvered Walnut tree. Last autumn I found a Sycamore tree in the midst of a wood at Gamlingay which shewed unmistakeable signs of silvering. This tree had been cut down and the shoots arising from the stool were silvered. Mr A. W. Hill has given me information of a Horse Chestnut tree at Kew which bears silvered foliage. The tree had been lopped and shoots which subsequently developed near the wounded surface became silvered. Mr Hill has also told me of a silvered Philadelphus (commonly called Syringa) which he recently saw growing in a garden at Henley-on-Thames. It is thus seen that silvering of the foliage is a phenomenon which occurs in plants of very different relationships.

In Cambridgeshire the fructifications of *Stereum purpureum* are present in abundance on Plum trees which have died after being silvered. It is by no means rare to find a single dead branch which bears the fructifications of this fungus, the rest of the tree carrying silvered foliage. My observations shew that the sporophores appear always on dead tissues and the thought has often occurred to one that perhaps these fruit-bodies only develop after death has been caused by some other agent. However, the evidence against this view is strong, and particularly strong, I think, when the inoculation experiments to be described below are considered. It is by no means remarkable for the fructifications of such a fungus not to develop until the tissues of the host are dead; for in the case of a large number of fungi that attack trees, the spore-bodies are produced only on dead portions of the host. It sometimes takes a considerable time for the sporophores of *Stereum purpureum* to develop on the branches of silvered trees which on casual examination appear to be dead. Thus a branch which apparently dies one season may not develop fructifications until the following year; I say *apparently* dies because a branch, though externally lifeless, may still retain a large amount of living tissue; and as long as there is abundant opportunity for the fungus to invade tissues hitherto unattacked, it is easy to understand why fruit-bodies are not produced. Hence in those orchards where the wise practice of cutting out branches apparently dead is followed, but few sporophores are likely to develop.

Sporophores of *Stereum purpureum* have been seen on dead portions

of silvered Apple trees, and in the case of two of the three silvered Red Currant bushes already seen by myself this *Stereum* was growing on dead tissue at the base of the bushes. On portions of the stool of the silvered Sycamore tree to which reference has previously been made, *Stereum purpureum* was growing, but in this connection it should be stated that similar stools of Birch close by and on which this fungus was also found bore shoots with normal foliage.

My own inoculation experiments in connection with this disease will now be described. They may be grouped under three heads according to the material used:

(1) Inoculations with natural sporophores of *Stereum purpureum.*

(2) Inoculations with the mycelium of *Stereum purpureum* grown from spores under conditions of pure culture.

(3) Inoculations with the spores of *Stereum purpureum.*

In all cases the sporophores used either directly or for the purpose of obtaining spores had been obtained from Plum trees.

1. *Inoculations with natural sporophores of "Stereum purpureum."*

It is not necessary to describe in detail the method of these inoculations and their results, because they are identical with those of Percival and Spencer Pickering. The inoculations were made both in old and young trees (two years old) of the Victoria and Czar varieties of Plum, pieces of damp sporophore being placed in T-shaped wounds either in side branches or in the main stem, the wounds being subsequently covered with tinfoil and bound with wool. Almost invariably such inoculations have been followed by silvering. Thus out of one series of 15 inoculations made in young trees last February no less than 12 resulted in silvering by the middle of May. One characteristic of these inoculations is the profuse gumming which takes place in the neighbourhood of the wound, the masses of gum being sometimes half an inch in diameter. However, the readiness of Plum trees to gum under injury is well known, and doubtless other organisms besides *Stereum purpureum* will induce the same reaction. If a branch is cut off a few weeks after such inoculation and examined, it is found that a part of the wood is discoloured a dark brown for a considerable distance on either side of the wound. Sections made of the discoloured portions in the region of inoculation shew abundant hyphae in the water-conducting elements which also contain large

masses of gummy matter. The discolouration of the wood is due to the accumulation of gum in these channels. No hyphae have yet been found at the extremities of the discoloured region, so it appears that the formation of gum occurs beyond the point to which the hyphae have attained. It is noticeable that the bark immediately outside the discoloured wood of such a branch remains apparently unaffected. Though one does not wish to lay undue stress on the characters of the mycelium present in the diseased portions of the wood, one may say nevertheless that the hyphae are identical in appearance with those found in the vessels of branches of Plum trees on which fructifications of *Stereum purpureum* are growing. These hyphae are narrow, septate, and run longitudinally in the water-conducting elements of the wood; on treatment with Delafield's Haematoxylin they are characterised by the presence of small deeply-staining granules. The fact that mycelium is found both below and above the point of inoculation shews that it travels in both directions. This is of importance in connection with the spread of the disease from one branch to another. In such inoculations the first leaves to become silvered are sometimes situated a considerable distance above the wound, whereas leaves in closer proximity to it may remain normal for some time longer.

A few inoculations of the branches of a Laburnum tree with pieces of sporophore derived from Plum resulted in silvering in each case. This confirms Pickering's conclusion that in all probability there is nothing in the nature of specialised parasitism in the mode of life of this fungus.

Several series of control experiments were performed in connection with the above inoculations. In one series, wounds were made as before but nothing was inserted in them. In another, pieces of sporophore of the fungus which had been killed by boiling for 5 minutes on each of three previous days were inserted in the wounds. In other series, pieces of the fructifications of *Stereum hirsutum* and of *Polystictus hirsutus* were used instead of *Stereum purpureum*. No sign of silvering resulted in any of the trees subjected to these various kinds of treatment. The control inoculations with *Stereum hirsutum* are of particular interest because this fungus is very closely allied to *Stereum purpureum*.

A few inoculations were performed with extracts of the fructifications of *Stereum purpureum*, obtained by boiling them in water; these experiments gave negative results. However, nothing can be safely deduced from these results.

2. *Inoculations with the mycelium of "Stereum purpureum" grown from spores under conditions of pure culture.*

Soon after these investigations had been begun it was found easy to cultivate *Stereum purpureum* from spores in the same way as Marshall Ward (6) had grown *Stereum hirsutum* some years ago. Inoculations made with sporophores taken from a dead branch are not free from suspicion of contamination by some other organism, and it is with such sporophores that all previous inoculation experiments have been performed. No such objection can be raised against the use of mycelium cultivated in pure culture from spores, so this mycelium has been used for a number of experiments.

On moistening natural sporophores of *Stereum purpureum* and placing them over clean glass slides in such a way as to prevent the fructifications from coming into contact with the slides, spores are deposited in quantity and can be transferred to sterilised blocks of Plum wood arranged in suitable tubes. In a few weeks a dense mycelial growth is obtained which can be used for purposes of inoculation. The mycelium of the fungus grown in this manner is at first white in colour, but subsequently becomes tawny. Small sporophores are produced in these artificial cultures, but they lack the firmness and definite form of sporophores which develop in nature. The inoculations with the mycelium grown in pure culture were made in the same way as those in which natural sporophores were used. It should be stated that the scalpel was always sterilised before a wound was made and again before using it to transfer the mycelium from the culture tube to the wound. Last autumn six such inoculations were made in the branches of a healthy Victoria Plum tree of mature age and this spring each one of these branches was found to bear silvered foliage. Between January and March some of the young trees planted last autumn were used for a similar series of inoculations. Thus 38 inoculations were made, some in Victorias and some in Czars. By the end of May, seven of the inoculated branches on three different trees shewed silvering of the leaves, and by the middle of June this number had increased to ten. The control branches shewed no sign of silvering. As time proceeds it is highly probable that more of the inoculated branches will become silvered. The amount of the fungus placed in the wound is less when pure cultures are employed than when natural sporophores are used, and this probably accounts for the relatively smaller effect of the cultivated mycelium to date.

It is to be noted, however, that where this mycelium had the whole of the winter in which to develop, silvering resulted the following spring in every case.

One of the branches which became silvered after treatment of this kind was cut off in order to try to trace the mycelium in the tissues from the place of inoculation. In the immediate neighbourhood of the wound a cross-section shows that a sector of about 80 degrees of the wood from pith to cambium is stained a deep brown. Such wood contains numerous hyphae and large quantities of gum are present in the water-conducting elements. These hyphae are similar to those described as being present in the earlier series of inoculations. A cross-section taken about 1½ inches above the wound shows a discoloured sector of about 50 degrees, but this dark brown area does not reach quite as far as the cambium. Hyphae are found in the vessels of this region also. Three and a half inches above the wound the affected region is limited to a narrow zone immediately surrounding the pith, which also is somewhat discoloured. So far I have not been able to determine the presence of hyphae in the wood at this level. Higher still, at 6 inches above the place of inoculation, the appearance is the same as that presented at the previous level. In this region the discoloured portion passed out to a small branch which was devoid of leaves. No hyphae could be seen at this level. The portion of the stem about 3½ inches below the wound will now be described. In transverse sections the dark brown area is still found to be present but is more irregular in shape than at higher levels; hyphae are present in this discoloured zone. A lateral branch attached just above this level showed no signs of discolouration in cross-section, although it bore silvered foliage. Such was found to be the case with other lateral branches attached to the inoculated stem. It is evident from these observations that the mycelium of *Stereum purpureum* extends more rapidly in a longitudinal than in a transverse direction and that silvering of the leaves occurs at levels to which the mycelium itself has not yet reached.

A similar series of observations has been made on the branch of a young Czar Plum tree, which was inoculated with cultivated mycelium at the end of March and showed silvering of the foliage in June. In this case the discoloured zone of wood extended to a distance about 1½ inches above the point of inoculation, but silvered leaves were found at a level more than a foot above the limit of discolouration.

3. *Inoculations with the spores of "Stereum purpureum."*

As far as I am aware, previous workers on this disease have made no inoculations with the spores of this fungus. To do so is a matter of importance, because if *Stereum purpureum* is the cause of Silver-leaf, infection is probably carried by means of these spores. During the last two years I have been making inoculations of Plum trees with the spores, but until recently with apparently no success. Nor would it have been surprising if efforts along these lines had been baffled indefinitely, because it is a matter of considerable difficulty to cause the infection of a tree with the spores of any of the higher wood-destroying fungi. Thus no one has yet successfully infected a tree with the spores of *Polyporus squamosus*, one of the commonest fungi of this class, and which in all probability is carried from tree to tree solely by means of spores.

After the lapse of a year, two of the twenty-one branches of fully grown Plum trees which last year were inoculated with the spores of *Stereum purpureum* now show conspicuous silvering, the successful inoculations being on two different trees. All the uninoculated branches of these trees show no signs of silvering, so in spite of these experiments being performed by necessity in a plantation containing other silvered trees some distance away, there are good grounds for supposing that silvering in these cases followed directly from the spore inoculations.

The number of such successes as yet obtained is very small, but bearing in mind the risk of failure attached to these inoculations, and also in view of the observations recorded below, the successes are significant.

These spore inoculations were performed in the same way as the other inoculations, except that spores only were inserted into the wounds. The spores were found to germinate fairly readily in control experiments under laboratory conditions.

One of the two branches, which already show silvering after inoculation with spores, has been cut off and investigated in the same manner as branches in which silvering has been induced by inoculation with mycelium cultivated in pure culture. Thus at the point of inoculation, where gumming had evidently occurred, there was a zone of wood deeply discoloured occupying a position about midway between pith and cambium and covering about 90 degrees of arc. This zone of dark brown wood was traced in a vertical plane

to levels about 4 inches above, and ¾ of an inch below, the plane of inoculation. Longitudinal sections showed the presence of the same kind of hyphae in the discoloured parts of the wood as those present in affected portions of the wood referred to in other series of inoculations. I think it may reasonably be concluded that these hyphae developed from the spores of *Stereum purpureum* originally inserted into the wound. Accumulations of gum similar to those already described were present in the water-conducting elements. Leaves 9 inches above the last trace of discoloured wood showed conspicuous silvering. On the inoculated stem, and attached between the upper and lower limits of the affected wood, a small lateral branch bore only healthy foliage. No discoloured wood was found on examining sections of this small branch.

On the young Plum trees which were planted in the experimental plot last autumn I have already performed a number of spore inoculations, but it is too early to speak of their results. The two successful spore inoculations described above had been made more than a year before silvering could be observed. But in order to see whether mycelium could be already traced in these spore inoculations of young trees one branch has been cut off and examined. At the point of inoculation a sector of dark brown wood extending over about 90 degrees was present, and in a vertical direction the zone of affected tissue stretched ¾ of an inch above, and 2 inches below, this level. Hyphae like those described above were seen in the vessels and gum was present in quantity. It is thus clear that the preliminary effects are in this case the same as in other inoculations which have eventually resulted in silvering. One may therefore expect a branch inoculated in such a manner to bear silvered foliage at a later date.

The results of all these experiments strengthen the view that *Stereum purpureum* is an active agent in inducing Silver-leaf disease in this country, and in my own mind they amount to proof that this fungus is a cause of it. It would be rash to say that *Stereum purpureum* is the *only* cause of Silver-leaf, but after considerable experience of the disease I cannot avoid the conclusion, shared of course by others, that this fungus is the agent chiefly responsible for the havoc wrought by the malady in the fruit-growing districts of this country.

The possible ways in which infection occurs in nature will now be considered. When Percival (3) first suggested that *Stereum purpureum* was the cause of the disease he considered that the roots were the

organs of the tree to be first attacked. However, in view of the distribution of the disease referred to at the beginning of this paper, and of the fact that frequently the suckers thrown up from the roots are the last parts to become silvered, it is doubtful if infection usually takes place in the roots. Furthermore, when Silver-leaf first appears in a particular area it is usually present on trees scattered here and there throughout the plantation; for several years the disease appears to spread indiscriminately, *e.g.* trees subsequently affected often bear no definite relationship in position to those originally attacked. If, on the other hand, the disease usually spread in a subterranean manner, one would naturally expect healthy trees close to silvered ones to become infected before others at a greater distance away. With Spencer Pickering, I incline rather to the view that infection more frequently occurs above ground and at some previously wounded surface. The fact that spore inoculations have been successful supports this view. Another circumstance favouring this opinion is the frequent appearance of a single silvered branch when a tree is first attacked. There is never any lack of wounds on which the spores of the fungus may germinate; pruning wounds not occluded by a callus are innumerable, and these are added to by the frequent splitting of the branches when pickers are gathering the fruit. Once the mycelium has entered the tissues it works both upwards and downwards and sooner or later enters the roots. Having entered the subterranean organs it may possibly spread through the soil to the roots of a healthy tree, but as yet there is no proof of this. Recently I saw a silvered Victoria Plum tree, nearly dead, from the bark of which at soil level a whitish mycelium was growing into the ground. It was not possible to trace this mycelium for more than a foot into the soil, and of course one cannot be certain that this mycelium belonged to *Stereum purpureum*.

A feature of the disease is the relatively long time which elapses between the first appearance of silvering and the death of the tree. This can be accounted for by the less rapid advance of the mycelium in a lateral than in a longitudinal direction; hence one side of a tree may remain healthy for some time after the other side is seriously affected. If, on the other hand, the mycelium spread rapidly in a lateral direction, all conduction of water would cease in the parts attacked, and death would soon ensue.

The occasional recovery of silvered trees has already been mentioned. I have seen a few Plum trees which, after showing conspicuous

silvering one year, have exhibited only the slightest traces of silvering the following year. This recovery may be looked upon as the victory of the host over the fungus, for until the end comes the struggle is continually being waged within the tissues of the host. But when a tree is very badly affected it is doubtful whether there is any chance of recovery. The percentage of silvered trees which regain complete health is very small and is almost negligible from the practical standpoint.

Where a single branch of a tree is silvered, endeavour should be made to cut out this limb as far back as the limit of the discoloured wood. It is useless, however, to cut away only the parts bearing silvered foliage without taking care that the branches have been severed below the lowest limit of diseased wood, for it has been shown above that the part of the wood containing the mycelium of the fungus is often a considerable distance below the foliage that is silvered. By such an operation there is a chance of saving the tree. It is obvious that where a large branch is cut away the exposed surface should be covered with a coating of tar. As soon as a silvered tree begins to die back it is advisable to remove it, for it is courting disaster to allow such a tree to remain *in situ* after death, and thereby afford opportunity for the production of the sporophores of *Stereum purpureum*. Even when trouble is taken to remove trees, they are often piled in the plantation, there to remain sufficiently long to allow of the formation of the fruit-bodies of this fungus. This practice is almost as bad as that of allowing dead trees to remain standing in the plantation. A short time ago I saw in a plantation of Plum trees a fence the posts of which were made of Plum wood. Sporophores of *Stereum purpureum* were growing in enormous numbers on these posts, and one can imagine the facility thereby afforded for the spread of Silver-leaf disease, which indeed had already made its appearance in the plantation.

The capacity of even a single sporophore of *Stereum purpureum* to produce spores is very large; furthermore, these sporophores possess great vitality, for they can be kept dry for weeks and even months, and upon being moistened will give rise to spores again within a few hours. The sporophores of *Stereum purpureum* in this country are to be found all the year round. The fungus is such a common saprophyte that it is impossible to eradicate it, but every means should be taken along the lines indicated above to prevent it from producing spores in fruit plantations.

At an early date I hope to publish another paper giving fuller details of the action of the mycelium of *Stereum purpureum* on Plum wood. Investigations are also being made upon the *modus operandi* of silvering and upon possible means of curative treatment, though I am afraid the outlook for the latter does not appear to be very promising at present.

Summary.

1. The distribution of Silver-leaf disease is described.

2. Inoculations of branches of Plum trees with sporophores of *Stereum purpureum* have almost invariably resulted in silvering.

3. The mycelium of *Stereum purpureum* has been cultivated in pure culture from spores and inoculations with this mycelium have caused silvering.

4. A small number of inoculations with the spores of *Stereum purpureum* have resulted in silvering.

5. Various series of control experiments have given negative results.

6. The investigations strengthen the view that *Stereum purpureum* is the active agent which causes Silver-leaf disease in this country.

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